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***Research Note***

# Lack of Transmammary Transmission of *Strongyloides stercoralis* from a Previously Hyperinfected Bitch to Her Pups

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ABSTRACT: Larvae were not found by Baermann examination of the gastrointestinal tract or parenteral tissues of 7-day-old pups whelped by a bitch previously hyperinfected with *Strongyloides stercoralis* and either suckled on the bitch or fed an artificial milk diet. In sharp contrast, experimentally infected comparable pups yielded larvae when examined by the same technique. Additionally, although the bitch transiently shed larvae in the feces prior to whelping, larvae were not found in filtered aliquots of the bitch's milk from day 1 to day 7 after whelping, whereas they were recovered from filtered aliquots of *S. stercoralis*–seeded milk samples. These results suggest that transmammary and transplacental transmission of the parasite does not occur in dogs.

KEY WORDS: *Strongyloides stercoralis,* transmammary transmission, canine model.

*Strongyloides stercoralis* is a nematode parasite with the ability to replicate within its host allowing for accumulation of adult and larval forms, both in the gastrointestinal tract and in extraintestinal sites. It infects humans, primates, and dogs and is capable of causing serious disease leading to death in all of these hosts. The most severe manifestations of strongyloidiasis are hyper- and disseminated infection in which precociously developing third-stage larvae autoinfect the host, leading to massive numbers of, respectively, enteral and parenteral forms. *Strongyloides stercoralis*–infected dogs treated with corticosteroids have been shown to develop both hyper- and disseminated infections with the parasite (Grove et al., 1983; Schad et al., 1984; Genta et al., 1986; Mansfield and Schad, 1992) providing a good model for the study of the human disease. Infections by species of strongyloides are usually acquired by either of 2 routes: by direct penetration of the skin in unsanitary surroundings or by transmammary transmission.

Because transmammary transmission occurs in several other *Strongyloides* species, it has been suspected to occur in *S. stercoralis,* but this was never proven. Vertical transmission of other *Strongyloides* species in animals has been well documented, including *Strongyloides ransomi* in pigs (Moncol and Batte, 1966; Batte and Moncol, 1968), *Strongyloides westeri* in horses (Lyons et al., 1969), *Strongyloides papillosus* in sheep and cattle (Lyons et al., 1970), and *Strongyloides ratti* in rats (Katz, 1969; Zamirdin and Wilson, 1974). Additionally, reports have described the presence of infective larvae in the milk of *Strongyloides* spp. (*Strongyloides papillosus* group) (Grove, 1989) and *Strongyloides fulleborni*–infected women (Brown and Girardeau, 1976). Vertical transmission of *S. fulleborni* also has been shown to occur in primates (Wong and Conrad, 1978) and is suspected in humans in Papua, New Guinea (Ashford and Barnish, 1989). Conflict exists, however, in distinguishing the species of *Strongyloides* and, in particular, in differentiating *S. stercoralis* from *S. fulleborni* in both human and primate infections. The identity of

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the single larva discovered in the Brown and Girardeau (1976) study was challenged (Grove, 1989) and claimed to be an autoinfective *S. stercoralis* larva. The possible occurrence of vertical transmission of *S. stercoralis* has greater implications for the host than in the other host–parasite associations because once an infection is acquired the parasite can increase its numbers within the host by autoinfection.

Transmammary transmission has been demonstrated in rats infected with *S. stercoralis* during pregnancy (Wilson et al., 1982). It is epidemiologically more relevant, however, to examine this phenomenon in previously infected females with cryptic or chronic infections because the majority of humans become infected early in life (Grove, 1989). Because we had a well-defined, laboratory-maintained strain of *S. stercoralis,* we undertook to test the hypothesis that the parasite can be transmitted vertically from chronically infected mother to offspring in dogs.

A laboratory-reared beagle bitch, negative for nematode parasites by repeated fecal flotation and Baermann examinations, was infected with 2,500 third-stage, infective larvae of *S. stercoralis* by subcutaneous injection. She developed a patent infection that was enhanced by prednisolone treatment (3.3 mg/kg, once a day). During the prednisolone treatment, the bitch was anesthetized with Thiopental (2.2 mg/kg intravenously, to effect), an endotracheal tube was passed, and 10 ml of sterile saline was flushed into and aspirated out of the lungs. The saline was examined at 10× magnification for the presence of larval stages of the parasite. The prednisolone was discontinued by tapering the dose gradually. Larval shedding ceased after 2 wk. After 4 mo with no steroid treatment, the bitch cycled and was bred by artificial insemination to a uninfected beagle dog. She conceived. At 35 days of gestation the 3 times/wk, fecal Baermann examinations were resumed. The bitch's temperature was recorded twice daily beginning at 50 days of gestation. When her temperature dropped to 37°C, she was watched closely for whelping. The bitch's perineal region was scrubbed with an iodine preparation to kill any existing *S. stercoralis* larvae, and she was prevented from having contact with her fecal material. To minimize the possibility of accidental reinfection of the bitch or infection of the pups by skin penetration, the bitch was housed in a stainless steel cage that was washed daily in a cage washer at 82°C. Six pups were received on sterile towels. Three pups were placed with the bitch for nursing, and 3 were taken to a separate incubator and fed a canine milk substitute (Esbilac, Borden Co., Hamshire, Illinois). From day 1 to day 7 after whelping, the bitch was milked once a day from all teats, a minimum of 10 ml of milk filtered through a 0.45-$\mu$m filter and the filter examined under 30× magnification for the presence of larvae of *S. stercoralis.* Bovine milk samples containing known quantities of third-stage larvae (10, 100, 1,000) were filtered by identical means and examined microscopically as controls. At 1 wk of age, all pups were sacrificed with an overdose of barbiturate anesthesia. All tissues except for the skin and gastrointestinal tract were examined during necropsy, divided into organ systems, minced, and soaked in phosphate-buffered saline (PBS) on screens (Baermann examination) for parasite recovery. The gastrointestinal tract was slit longitudinally and hung in graduated cylinders containing PBS and the skin was soaked in a flat pan of PBS, after parallel partial-thickness slicing of the subcutaneous side of the skin (Bianco et al., 1980). All preparations were incubated at 37°C for 3 hr, the tissue removed, and the solid material sedimented and searched for larvae of the parasite. Feces taken from the rectum of the pups during necropsy was subjected to Baermann examination. Three 10-day-old purpose-bred, parasite-naive control pups were preanesthetized with Innovar® (0.4 mg/9–22.5 kg fentanyl and 50 mg/9–22.5 kg droperidol, subcutaneous), anesthetized with 2% halothane delivered via mask with oxygen, and infected by inoculation of approximately 1,500 third-stage larvae through a ventral midline abdominal incision directly into the exteriorized distal ileum. The pups were allowed to recover from anesthesia and were examined by identical means at approximately 4 days after infection to test our ability to recover migrating larvae.

The bitch developed a patent infection in approximately 17 days, shed larvae in the feces for a 30-day period, and then ceased to shed larvae as determined by 3 times/wk fecal Baermann examinations (Fig. 1). After 3 consecutive negative Baermann examinations, the dog was given oral prednisolone. Seven days after commencing drug treatment, the dog shed first-stage larvae of the parasite in the feces, the numbers of which continued to increase over a 6-wk period (Fig.

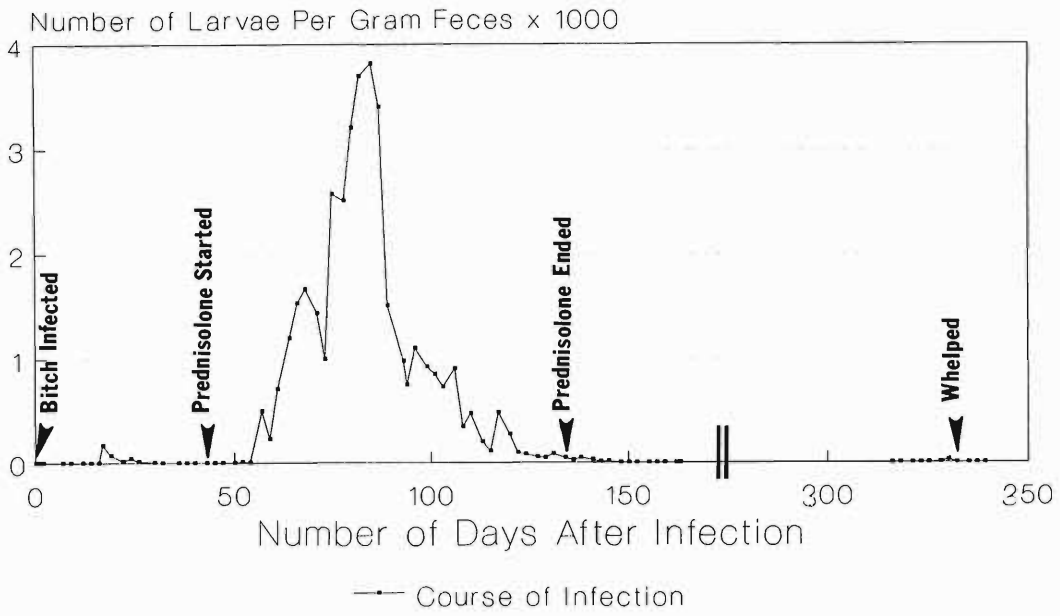


**Figure 1. The course of infection of a *Strongyloides stercoralis*–infected bitch treated with oral prednisolone to induce hyperinfection.**

1). At the peak of larval shedding, the bitch excreted 3,800 larvae/g in a 50.0-g sample. This exceeded the maximum first-stage larval yield for *Strongyloides* spp., which may be determined by multiplying the infective dose by the maximum number of eggs/adult/day (estimated maximums of 40) (Triantaphyllou and Moncol, 1977). This confirmed the occurrence of hyperinfection, because a greater number of shedding adult females would have been required to produce the observed number of fecal larvae than the number given in the infective dose. Additionally, 2 third-stage larvae were recovered by tracheal wash, indicating the presence of the parasite outside the gastrointestinal tract after the prepatent period, suggesting active larval migration, although simple aberrant larval location cannot be ruled out.

**Table 1. Number of *Strongyloides stercoralis* parasites recovered by quantitative Baermann examination from 7-day-old pups whelped by previously hyperinfected bitch and control pups infected with 1,500 third-stage larvae. Experimental pups were either separated from the bitch at whelping or allowed to suckle on the bitch.**

| | Pup number | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| Group 1—No suckling | | | |
| Skin | 0 | 0 | 0 |
| Gastrointestinal tract | 0 | 0 | 0 |
| All other organs | 0 | 0 | 0 |
| Group 2—Suckling | | | |
| Skin | 0 | 0 | 0 |
| Gastrointestinal tract | 0 | 0 | 0 |
| All other organs | 0 | 0 | 0 |
| Group 3—Control* | | | |
| Skin | 0 | 0 | 2 |
| Gastrointestinal tract | 99 | 30 | 220 |
| All other organs | 4 | 0 | 2 |

* Ten-day-old pups were experimentally infected with 1,500 third-stage *Strongyloides stercoralis* larvae.

The bitch shed first-stage larvae in the feces intermittently at low levels (1–10 larvae/g) in the 2 wk prior to whelping but ceased to shed larvae from the day of whelping and thereafter (Fig. 1). Milk was produced by the bitch from the day of whelping until the conclusion of the experiment. The milk was examined for the presence of larvae from day 1 to day 7 after whelping. No larvae were found in the milk of the bitch at any time during the study, whereas control milk samples seeded with larvae were judged positive by the filter screening procedure. No larvae were found in the feces, gastrointestinal tract, skin, or internal organs of any of the pups whelped by the bitch (Table 1). In contrast, larvae were recovered from control pups experimentally infected with the parasite and examined by identical means (Table 1).

Transmammary or transplacental transmission of *S. stercoralis* in humans has not been documented conclusively at this time. Our results suggest that vertical transmission of the parasite does not occur in dogs. The bitch described in this study harbored a patent infection of *S. stercoralis*. She developed a hyperinfection of the parasite during corticosteroid therapy as judged from fecal larval recoveries higher than possible from the infecting dose alone and the presence of third-stage larvae in the lungs after the prepatent period. Despite the chronic-active nature of her infection, the bitch failed to transmit any detectable larvae to her offspring, either through the placenta or through the milk. The 3 pups allowed to suckle on the bitch served as a test for transmission of larvae via the transmammary route, whereas the 3 pups fed an artificial milk replacer served as a test for transmission of larvae via the transplacental route. Although recovering larvae from milk is difficult (Ashford and Barnish, 1989), the use of pups to suckle milk, and thereby collect any larvae present and concentrate them in their guts, should maximize recoveries. Transmammary transmission of *Strongyloides* spp. may depend on the general susceptibility of the host and the timing of the infection. Sows transmit *S. ransomi* to their offspring in the milk from tissue stores (Moncol and Batte, 1966; Batte and Moncol, 1968), but rats must be infected during gestation to pass *S. ratti* in the milk (Zamirdin and Wilson, 1974; Wilson et al., 1982).

The lack of transmammary passage of *S. stercoralis* from this experimental bitch to her pups casts doubt on the speculation that transmammary transmission of this parasite occurs in humans or other natural hosts. However, because this experiment involved only 1 dog, the results are merely suggestive, but they should serve as a preliminary study on which additional studies of vertical transmission in *S. stercoralis*–infected animal models (dog and primate) could be based.

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